Colonization of the Human Oral Cavity by a Streptococcus mutans Mutant Producing Increased Bacteriocin

J.D. Hillman, A.L. Dzuback and S.W. Andrews

J DENT RES 1987 66: 1092
DOI: 10.1177/00220345870660060101

Colonization of the Human Oral Cavity by a *Streptococcus mutans* Mutant Producing Increased Bacteriocin

J.D. HILLMAN, A.L. DZUBACK, and S.W. ANDREWS

Department of Molecular Genetics, Forsyth Dental Center, 140 Fenway, Boston, Massachusetts 02115

Streptococcus mutans *strain JH1005 is a mutant that produces levels of bacteriocin activity three-fold-elevated than those produced by its parent, JH1001. A single infection regimen with JH1005 was found to result in persistent colonization of the teeth of all three adult subjects tested. This is a significant improvement over JH1001, which required multiple exposures in order to colonize the teeth of humans reliably. The levels of total cultivable bacteria and indigenous* S. sanguis *were not affected by JH1005 colonization. In two of the three subjects, total (indigenous plus JH1005)* S. mutans *levels were significantly decreased. The results provide additional support for the role of bacteriocin production as an ecological determinant in colonization by* S. mutans. *They also indicate that a practical regimen for infection by an effector strain might be achieved for use in the replacement therapy of dental caries.*

J Dent Res 66(6):1092–1094, June, 1987

Introduction.

The application of replacement therapy to the prevention and treatment of bacterial infections has certain potential advantages over more conventional therapeutic approaches. Ideally, a single application of the effector strain to the tissues at risk should result in its persistent colonization at levels sufficient to protect the host from infection by a pathogen. By becoming part of the normal flora of the host, a well-designed effector strain might provide life-long protection against a disease while requiring minimum compliance, education, or cost on the part of the subject. This approach also has the potential advantage of providing herd protection by the natural transmission of the effector strain within the population.

The application of replacement therapy to dental caries has been limited by the ability to obtain a strain of *S. mutans* that can superinfect the teeth of subjects who already harbor an indigenous strain of this organism (Krasse *et al.*, 1967; Jordan *et al.*, 1972; Edman *et al.*, 1975; Ruangsri and Ørstavik, 1977; Svanberg and Loesche, 1978; Svanberg and Krasse, 1981; Tanzer *et al.*, 1982). We (Hillman *et al.*, 1984) recently reported the isolation of a serogroup *c* strain of *S. mutans* called JH1001 that demonstrated unusually good colonization potential in rodents. It was shown that the ability of this strain to colonize the oral cavity correlated with its production of a potent, broad-spectrum bacteriocin. In a separate study (Hillman *et al.*, 1985b), repeated exposure of human subjects to JH1001 was found to result in prolonged (three-year) colonization by this strain, with partial to complete displacement of the subjects' indigenous *S. mutans*.

Also reported was a mutant of strain JH1001, called JH1005, that makes approximately three-fold-elevated bacteriocin activity (Hillman *et al.*, 1984). This mutant was found to colonize the oral cavity of rodents and displace indigenous *S. mutans* more efficiently than does JH1001. In the present study, JH1005 was examined for its ability to colonize the human oral cavity and displace indigenous *S. mutans*.

Materials and methods.

Micro-organisms and media. — *S. mutans* strain JH1005 has been previously characterized (Hillman *et al.*, 1984). It is an ethylmethane sulfonate-induced mutant of serogroup *c* strain JH1001 (resistant to 1 μg/mL tetracycline) that makes approximately three-fold-elevated amounts of bacteriocin activity. Broth cultures of micro-organisms were incubated overnight in air at 37°C in Todd-Hewitt medium (Difco) with added (0.5%) glucose. *S. sanguis* and *S. mutans* levels in saliva samples were measured by mitis salivarius (MS) agar and mitis salivarius agar with bacitracin (MSB; Gold *et al.*, 1973), respectively. The total cultivable bacteria in saliva was measured with trypticase soy agar containing 5% sheep's blood.

Infection of subjects. — Three male staff volunteers of the Forsyth Dental Center served as subjects. Representative isolates of their indigenous *S. sanguis* and *S. mutans* were obtained from unstimulated saliva samples by their characteristic morphologies on MS and MSB media, respectively. The identities of these isolates were verified by means of a semi-automated technique for identification of plaque bacteria (Dzink *et al.*, 1984). Sensitivity of the isolates to the JH1005 bacteriocin was tested as described below.

Infection of subjects with JH1005 utilized the methods employed previously for infection with strain JH1001 (Hillman *et al.*, 1985b). A 100-mL overnight culture (*ca.* 10^9 colony-forming units/mL) was centrifuged and the cells re-suspended in 5 mL of Todd-Hewitt broth containing 2% sucrose. The cell suspension was kept on ice until use, within one hour of preparation. The subjects' teeth were polished with pumice and a rubber cup. The suspension of JH1005 was then flossed and

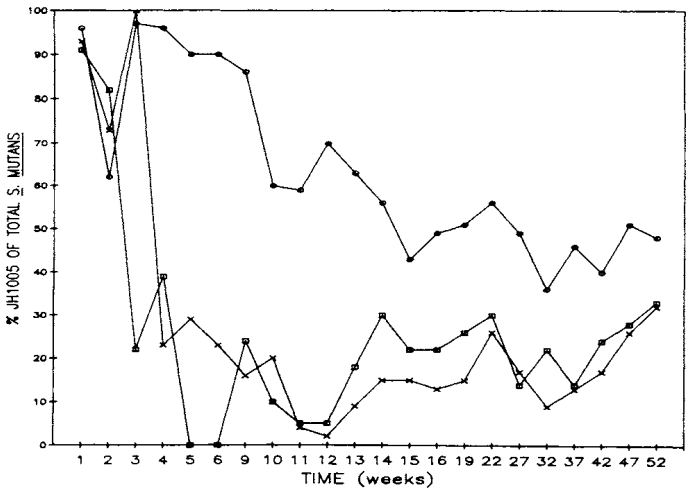

Fig. — The proportion of total *S. mutans* that was JH1005 is plotted against time for subject 1 (-x-), subject 2 (-o-), and subject 3 (-□-).

Received for publication November 4, 1986
Accepted for publication January 14, 1987
This investigation was supported by USPHS Research Grant DE04529 from the National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892.

TABLE

S. mutans, S. sanguis, AND TOTAL BACTERIA BEFORE AND ONE YEAR AFTER TREATMENT WITH JH1005

	Mean Concentration in Saliva*					
	Total *S. mutans* (CFU × 10^5/mL)		*S. sanguis* (CFU × 10^6/mL)		Total Bacteria (CFU × 10^8/mL)	
Subject	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
1	29.0	24.0	5.2	6.4	4.7	3.2
2	11.0	1.5**	3.6	4.0	2.9	1.7
3	23.0	0.6**	4.7	3.9	1.3	2.1

*Values are the means of nine pre-treatment and 22 post-treatment determinations.
**Significant ($p<0.001$) by the Mann-Whitney U Test.

brushed onto their teeth for three minutes. Unattached cells were removed by repeated rinses with water.

Analysis of saliva samples. — Prior to and following infection with JH1005, an amount (one to two mL) of unstimulated saliva was collected in sterile tubes at the indicated times and tested for its content of total cultivable bacteria, indigenous *S. sanguis* and *S. mutans*, and JH1005. The saliva was vortexed for 30 seconds and serially diluted in phosphate-buffered saline (pH 7). Aliquots were spread on blood agar medium, MS, and MSB, and incubated for 48 hr in candle jars at 37°C. Bacterial concentrations in saliva were determined by colony counts of plates containing from 50 to 250 colonies. Isolated colonies (50) arising on MSB with characteristic *S. mutans* morphology were picked at random and replica-patched onto MSB medium with and without 1 μg/mL tetracycline to distinguish indigenous *S. mutans* from JH1005. These data were corrected for differences between the indigenous strains and JH1005 in their plating efficiencies on MSB (Schaeken *et al.*, 1986) relative to blood agar medium.

Overlay assay for bacteriocin production and sensitivity. — Single colonies of strain JH1005 arising on isolation plates were stab-inoculated into brain-heart infusion agar (Difco), and incubated for 24 hr in candle jars at 37°C. Three mL of molten top agar containing *ca.* 10^6 colony-forming units/mL of the indicator strain was poured evenly over the surface of the plate. After an additional 24 hours of incubation, the diameters of the clear zones surrounding the JH1005 stabs were measured.

Results.

Indigenous *S. mutans* and *S. sanguis* were isolated from the saliva of three adult subjects and purified on the selective media. All of these isolates were found to be sensitive to 1 μg/mL tetracycline and were inhibited in the overlay assay by the JH1005 bacteriocin. Twenty-four-hour-old stabs of JH1005 caused clear zones in the lawns of the *S. mutans* and *S. sanguis* isolates, averaging 2.7 ± 0.4 cm and 2.5 ± 0.4 cm, respectively.

Baseline values for the concentrations of total cultivable bacteria and indigenous *S. mutans* and *S. sanguis* in saliva of the subjects were determined from nine successive weekly samples. Following infection with JH1005 by a single exposure to a concentrated cell suspension, saliva samples were analyzed for their microbial content at the indicated times (Fig.). Immediately following infection, JH1005 was found to constitute the majority of *S. mutans* recoverable from saliva in all three subjects. Within 3 to 4 weeks, the proportion of JH1005 to total *S. mutans* in subjects #1 and 3 dropped dramatically and then rose to values of approximately 30% by 52 weeks. The values for subject #2 remained relatively high throughout the experiment; at 52 weeks post-infection, JH1005 constituted approximately 50% of the total *S. mutans* in this subject.

At 9, 27, and 52 weeks post-infection, 20 indigenous *S. mutans* isolates from each subject were tested and shown by the overlay technique to have unaltered sensitivity to the JH1005 bacteriocin. Twenty JH1005 isolates obtained at the same times were unaltered in their abilities to produce the bacteriocin.

By three weeks post-infection, it was found that the mean total (indigenous plus JH1005) *S. mutans* levels had decreased approximately 7- and 38-fold in subjects #2 and 3 (Table). These values remained depressed throughout the remainder of the experiment. No changes in the total *S. mutans* levels were observed in subject #1.

Although the indigenous *S. sanguis* strains in these subjects were shown to be sensitive to the JH1005 bacteriocin, no significant changes in the levels of this organism were observed throughout the course of the experiment (Table). Similarly, the levels of total cultivable flora in saliva, as measured on blood agar medium, did not change as a result of infection with JH1005 (Table).

Discussion.

In a previous study (Hillman *et al.*, 1985b), it was found that human subjects required four or more exposures to strain JH1001 in order to assure persistent infection by it. In the present study, three similar subjects were colonized as the result of a single exposure to JH1005, a mutant of JH1001 that produces three-fold-elevated bacteriocin activity. These results agree with experiments performed in rodents (Hillman *et al.*, 1984) which demonstrated that superinfection potential correlated with *in vitro* levels of bacteriocin production.

As with JH1001, two general patterns were observed for the time-course of JH1005 colonization. Two of the three subjects showed a rapid decline in the proportion of JH1005 after infection, followed by a gradual increase. The third subject failed to show this initial rapid decline and maintained generally higher levels of JH1005 throughout the experiment. The basis for this difference is unknown; it does not appear to relate to differences in the levels of indigenous *S. mutans* or their sensitivity to the JH1005 bacteriocin. Nor were there apparent significant differences between the subjects with regard to oral hygiene.

Of interest was the observation that total *S. mutans* levels decreased significantly following infection with JH1005 in two of the three subjects (the third subject, who did not respond in this fashion, was later found to be participating in another study involving frequent exposures to high levels of other laboratory strains of *S. mutans*). The initial decline in these levels can be attributed to the dental prophylaxis which was performed on each subject prior to infection. However, the effects of dental prophylaxis on *S. mutans* levels is known to be short-lived (Caufield and Gibbons, 1979). The prolonged reduction in *S. mutans* levels was also not observed in subjects infected

with JH1001. This phenomenon, therefore, probably depends on the high levels of bacteriocin produced by JH1005.

Indigenous *S. sanguis* strains from the subjects were found to be sensitive to the JH1005 bacteriocin at levels comparable with those of their indigenous *S. mutans*. As in the case of infection with JH1001, the levels of *S. sanguis* did not change significantly throughout the course of the experiment. While both *S. sanguis* and *S. mutans* are known to colonize the crowns of teeth preferentially, this finding suggests that they have physically distinct, non-overlapping niches. The importance of this finding relates to recent evidence (Hillman *et al.*, 1985a) that *S. sanguis* is one of several organisms in plaque that may be essential to the maintenance of periodontal health. Successful application of replacement therapy to the prevention of dental caries must assure that the effector strain does not alter the ecological balance of plaque to the extent that the host becomes predisposed to other diseases.

REFERENCES

CAUFIELD, P.W. and GIBBONS, R.J. (1979): Suppression of *Streptococcus mutans* in the Mouths of Humans by a Dental Prophylaxis and Topically-applied Iodine, *J Dent Res* 58:1317–1326.

DZINK, J.L.; SMITH, C.; and SOCRANSKY, S.S. (1984): Semi-automated Technique for Identification of Subgingival Isolates, *J Clin Microbiol* 19:599–605.

EDMAN, D.C.; KEENE, H.S.; SHKLAIR, I.L.; and HOERMAN, K. (1975): Dental Floss for Implantation and Sampling of *Streptococcus mutans* From Approximal Surfaces of Human Teeth, *Arch Oral Biol* 20:145–148.

GOLD, O.G.; JORDAN, H.V.; and WHITE, G. (1973): A Selective Medium for *Streptococcus mutans, Arch Oral Biol* 18:1357–1364.

HILLMAN, J.D.; JOHNSON, K.P.; and YAPHE, B.I. (1984): Isolation of a *Streptococcus mutans* Strain Producing a Novel Bacteriocin, *Infect Immun* 44:141–144.

HILLMAN, J.D.; SOCRANSKY, S.S.; and SHIVERS, M. (1985a): The Relationships Between Streptococcal Species and Periodontopathic Bacteria in Human Dental Plaque, *Arch Oral Biol* 30:791–795.

HILLMAN, J.D.; YAPHE, B.I.; and JOHNSON, K.P. (1985b): Colonization of the Human Oral Cavity by a Strain of *Streptococcus mutans, J Dent Res* 64:1272–1274.

JORDAN, H.V.; ENGLANDER, H.R.; ENGLER, W.O.; and KULCZYK, S. (1972): Observations on the Implantation and Transmission of *Streptococcus mutans* in Humans, *J Dent Res* 51:515–518.

KRASSE, B.; EDWARDSSON, S.; SVENSSON, I.; and TRELL, L. (1967): Implantation of Caries-inducing Streptococci in the Human Oral Cavity, *Arch Oral Biol* 12:231–236.

RUANGSRI, P. and ØRSTAVIK, D. (1977): Effect of the Acquired Pellicle and of Dental Plaque on the Implantation of *Streptococcus mutans* on Tooth Surfaces in Man, *Caries Res* 11:204–210.

SCHAEKEN, M.J.M.; VAN DER HOEVEN, J.S.; and FRANKEN, H.C.M. (1986): Comparative Recovery of *Streptococcus mutans* on Five Isolation Media, Including a New Simple Selective Medium, *J Dent Res* 65:906–908.

SVANBERG, M. and KRASSE, B. (1981): Oral Implantation of Saliva-treated *Streptococcus mutans* in Man, *Arch Oral Biol* 26:197–201.

SVANBERG, M. and LOESCHE, W.J. (1978): Implantation of *Streptococcus mutans* on Tooth Surfaces in Man, *Arch Oral Biol* 23:551–556.

TANZER, J.M.; KRASSE, B.; and SVANBERG, M. (1982): Conditions for Implantation of *Streptococcus mutans* Mutant 805 in Adult Human Mouths, IADR *Progr & Abst* 61:No. 1400.